PTCH1 gene, which is implicated in susceptibility to different forms of skin cancer and carries three intronic variants, one of which affects transcription factor binding. Functional analysis of the possible roles of these and other variants in determining the MSSE phenotype, e.g., by gene expression or chromatin immunoprecipitation sequencing analysis to assess differential transcription factor binding, will require further studies using keratinocytes or cultured primary tumor cells from MSSE-affected patients. Finally, it is possible that, as loss-of-function germline mutations in *TGFBR1* are very rare, the conserved haplotype may act as a modifier to increase survival of individuals carrying strong mutations in this potent developmental regulator. This mechanism is supported by the identification of unlinked variant genetic modifiers that are preferentially inherited in mice haploinsufficient for Tgfb1 (Benzinou *et al*, 2012).

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

REFERENCES

Benzinou M, Clermont FF, Letteboer TG *et al.* (2012) Mouse and human strategies identify PTPN14 as a modifier of angiogenesis and hereditary haemorrhagic telangiectasia. *Nat Commun* 10:616

Bose S, Morgan LJ, Booth DR *et al.* (2006) The elusive multiple self-healing squamous epithelioma (MSSE) gene: further mapping, analysis of candidates, and loss of heterozygosity. *Oncogene* 25:806–12

D'Alessandro M, Coats SE, Morley SM *et al.* (2007) Multiple self-healing squamous epithelioma in different ethnic groups: more than a founder mutation disorder? *J Invest Dermatol* 127:2336–44

Ferguson-Smith J (1934) A case of multiple primary squamous-celled carcinomata in a young man, with spontaneous healing. *Br J Dermatol* 46:267

Goudie DR, D'Alessandro M, Merriman B *et al.* (2011) Multiple self-healing squamous epithelioma is caused by a disease-specific spectrum of mutations in TGFBR1. *Nat Genet* 43:365–9

Goudie DR, Yuille MA, Leversha MA *et al.* (1993) Multiple self-healing squamous epitheliomata (ESS1) mapped to chromosome 9q22-q31 in families with common ancestry. *Nat Genet* 3:165–9

Hahn H, Wicking C, Zaphiropoulous PG *et al.* (1996) Mutations of the human homolog of Drosophila patched in the nevoid basal cell carcinoma syndrome. *Cell* 85:841–51

Johnson RL, Rothman AL, Xie J *et al.* (1996) Human homolog of patched, a candidate gene for the basal cell nevus syndrome. *Science* 272:1668–71

Loeys BL, Chen J, Neptune ER *et al.* (2005) A syndrome of altered cardiovascular, craniofacial, neurocognitive and skeletal development caused by mutations in TGFBR1 or TGFBR2. *Nat Genet* 37:275–81

Sinha S, Singh RK, Alam N *et al.* (2008) Alterations in candidate genes PHF2, FANCC, PTCH1 and XPA at chromosomal 9q22.3 region: Pathological significance in early- and late-onset breast carcinoma. *Mol Cancer* 7:84

Wakabayashi Y, Mao JH, Brown K *et al.* (2007) Promotion of H-ras-induced squamous carcinomas by a polymorphic variant of the Patched gene in FVB mice. *Nature* 445:761–5

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Intravenously Injected Recombinant Human Type VII Collagen Homes to Skin Wounds and Restores Skin Integrity of Dystrophic Epidermolysis Bullosa

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TO THE EDITOR

Type VII collagen (C7) is located in the skin between its two main layers, the epidermis and the dermis, where it forms structures, anchoring fibrils (AFs), which are necessary for epidermal–dermal adherence (Sakai *et al.*, 1986; Burgeson, 1993). The *COL7A1* gene encodes for a 290-kDa alpha chain (Christiano *et al.*, 1994). C7 is composed of three alpha chains that form a homotrimer of ~900 kDa. Within the extracellular space, C7 molecules form antiparallel dimers, which aggregate laterally to form AFs. C7 has some unusual properties that make it quite

Abbreviations: AF, anchoring fibril; C7, type VII collagen; DEJ, dermal–epidermal junction; IV, intravenous; rC7, human recombinant C7; RDEB, recessive dystrophic epidermolysis bullosa

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distinctive from other collagens, such as its solubility in neutral buffers. Most skin-associated collagens such as type I collagen induce platelet aggregation and clot formation when exposed to the blood stream, whereas C7 does not (Saelman *et al.*, 1994).

Defects in both alleles of *COL7A1* cause an absence or reduction of functional C7 and AFs, resulting in recessive dystrophic epidermolysis bullosa (RDEB). RDEB patients have skin fragility, skin blistering, erosions, milia formation, nail loss, joint contractures, fibrotic mitten deformities of the hands and feet, mutilating scars, esophageal strictures, and aggressive squamous cell carcinomas that take the patients' lives prematurely (Fine *et al.*, 2008). Unfortunately, RDEB is incurable. Various therapeutic strategies have been envisioned for RDEB based on preclinical animal models, including the intradermal injection of allogeneic dermal fibroblasts or gene-corrected RDEB fibroblasts (Ortiz-Urda *et al.*, 2003; Woodley *et al.*, 2003), intradermal injection of lentiviral vectors expressing C7 (Woodley *et al.*, 2004), and transplantation of gene-corrected keratinocyte autografts (Chen *et al.*, 2002; Ortiz-Urda *et al.*, 2002). Recently, proof-of-principle clinical trials have been initiated in RDEB patients, including bone marrow/stem cell transplantation and intradermal injection of allogeneic fibroblasts (Wong *et al.*, 2008; Wagner *et al.*, 2010). None of these therapies have proven to be consistently effective.

We demonstrated that the intradermal injection of human recombinant C7 (rC7) into RDEB skin equivalents grafted onto immunodeficient mice or into RDEB-like, C7-knockout mice results in new C7 and AFs and reverses the RDEB skin phenotype (Woodley *et al.*, 2004; Remington *et al.*, 2009). Nevertheless, intradermally injected rC7 has a small diffusion radius and is not ideal for treating large areas of skin in RDEB patients who usually have widespread lesions, including the oral cavity and esophagus. In the present study, we sought to determine whether we could ameliorate RDEB by intravenously (IV) administering rC7. We hypothesized that IVC7 would simultaneously home to multiple RDEB wounds and restore C7 expression and function.

We used two animal models—a full-thickness skin wound created in athymic nude mice and an RDEB skin transplantation mouse model. For the first model, 1 cm by 1 cm full-thickness wounds were made on the backs of athymic nude mice. Between 4 and 8 hours later, we injected the tail veins of the mice with rC7. As shown in Figure 1a, the IV-injected rC7 homed

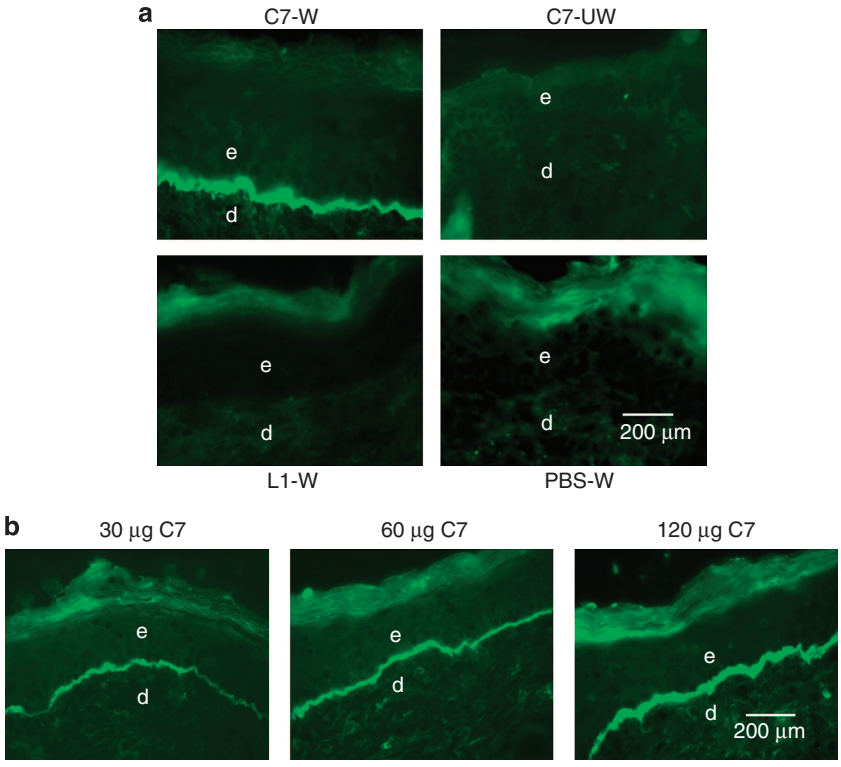

Figure 1. Intravenous (IV)-injected rC7 homes to skin wounds and incorporates into the mouse's regenerated dermal (d)–epidermal (e) junction (DEJ). (**a**) Immunofluorescence staining of mouse skin (after IV injection of 100 μg of rC7 or laminin 111) was performed with antibodies specific for human C7 at 2 weeks after the injection. Note that the IV-injected rC7 ($n = 20$ mice) homed and incorporated into the regenerated DEJ of the healed wound sites (C7-W) but was not present in unwounded skin sites (C7-UW). No rC7 was detected in mice IV injected with laminin 111 ($n = 5$ mice) or phosphate-buffered saline (PBS; $n = 10$ mice) (L1-W and PBS-W). (**b**) Dose-dependent deposition of rC7 at the mouse's DEJ after IV injection with rC7. Immunofluorescence staining with an antibody specific for human C7 was performed on healed mouse skin wounds 2 weeks after the animals were injected with 30 μg rC7, 60 μg rC7, or 120 μg rC7, as indicated, respectively. All photographs were taken at the same exposure time.

to wound sites and incorporated into the dermal–epidermal junction (DEJ) at 2 weeks after injection. By contrast, there was no detectable human rC7 in unwounded skin. None of the mice injected with PBS (phosphate-buffered saline) or laminin 111, a large non-collagenous glycoprotein within basement membranes, had rC7 in their healed skin. As shown in Figure 1b, with increasing doses of rC7, we detected a dose-dependent increase in rC7 at the mouse's DEJ. In addition, IV-injected rC7 was sustained at the mouse's DEJ for at least 8 weeks (data not shown).

We then examined whether IV-injected rC7 trafficked to other organs. Human rC7 was only observed in healed skin wounds and not in unwounded skin or esophagus, stomach, tongue, small intestine, brain, kidney, liver, lung, spleen, or heart (Supplementary Figure S1 online). Finally, we did not observe any adverse effects in mice receiving IV rC7 at doses as high as 33 mg per kg body weight. Daily weight, activity, and feeding habits were identical between the experimental and control groups.

To evaluate intravenous C7 in an RDEB-like model, we transplanted skin from C7 knockout mice that have no C7 or AFs in their DEJ (Heinonen *et al.*, 1999) onto the backs of athymic nude mice. These newborn, *COL7A1*-null mice exhibit extensive skin blisters and die within the first week of life. These C7-null mice recapitulate the clinical, genetic, and ultrastructural features of RDEB. Because these small neonatal mice only live for a few days, it is not technically feasible to inject their tail veins with C7. As an alternative, we kill these mice after birth and transplant their skin onto the backs of adult athymic, nude mice. As shown in Figure 2a, RDEB skin grafts before treatment showed histological evidence of dermal–epidermal separation and entirely lacked C7 staining at the DEJ, which is characteristic of RDEB.

Full-thickness skin wounds are very different from the superficial bullous wounds of RDEB skin, which occur at the epidermal–dermal interface. Having shown that intravenous rC7 homes to full-thickness skin wounds, we wished to evaluate whether intravenous rC7 would also home to grafted RDEB mouse skin wounds. We administered intravenous rC7 to the adult athymic nude mice hosting RDEB mouse skin grafts. As shown in Figure 2a, IV-injected rC7 homed to the RDEB mouse skin, incorporated into the DEJ, and corrected the dermal–epidermal separation. By contrast, C7-null RDEB-like skin transplanted onto mice that received PBS injections revealed dermal–epidermal separation and had no rC7. These experiments show that although the characteristic RDEB wound is superficial the wound is sufficient to allow intravenously injected rC7 to

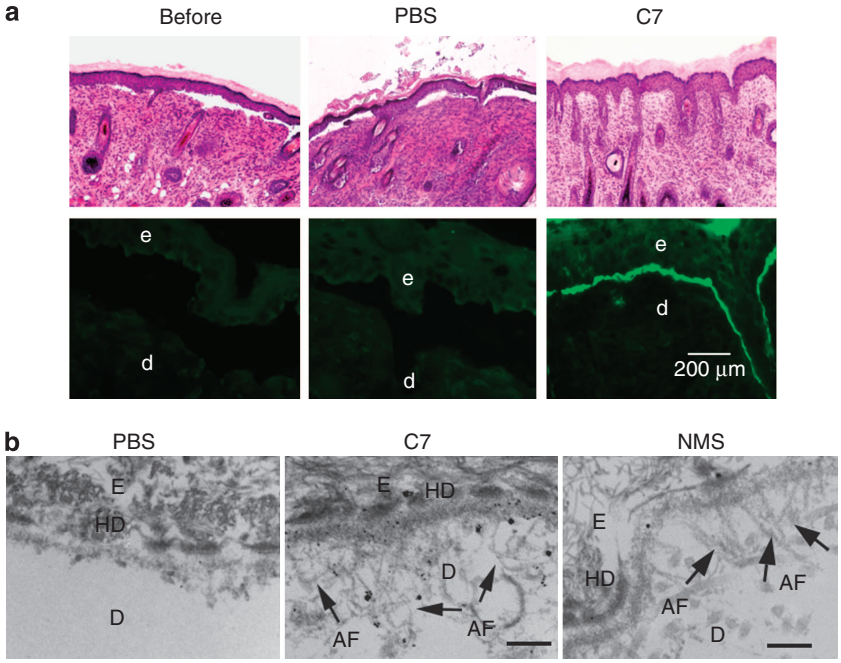

Figure 2. Intravenous (IV)-injected rC7 incorporates into the dermal–epidermal junction (DEJ) of recessive dystrophic epidermolysis bullosa (RDEB) mouse skin grafted onto athymic nude mice *in vivo*. (**a**) Histological appearance (upper panels) and immunofluorescence staining (lower panels) of engrafted RDEB mouse skin using a monoclonal antibody specific for human C7. Left panels (Before) are skin biopsies from transplanted RDEB skin grafts taken 2 weeks after grafting and before treatment ($n = 30$ mice). Middle panels (phosphate-buffered saline (PBS)) are 2-week post-injection biopsies from RDEB skin grafts on athymic host nude mice that were IV injected with PBS ($n = 4$ mice). Right panels (C7) are 2-week post-injection biopsies from RDEB skin grafts on athymic host nude mice that were IV injected with 60 µg of rC7 (n = 9 mice). e, epidermis; d, dermis. (**b**) Immunogold labeling of engrafted murine RDEB skin injected with PBS or 60 µg rC7 (C7) was performed with an antibody specific to human C7 (NP185, a gift of Dr Lynn Sakai, Shriners Hospital for Children, Portland, Oregon), followed by a 1-nm gold secondary antibody. Identical immuno-electron microscopy (IEM) was performed on normal mouse skin (NMS). Note that IV-injected rC7 incorporated into the RDEB skin grafts and formed anchoring fibrils (AFs). Note restoration of numerous arching AFs depicted with arrows and labeled with gold particles decorating the DEJ of RDEB skin grafts that received IV rC7. IEM on NMS shows numerous unlabeled AFs. D, dermis; E, epidermis; HD, hemidesmosomes; bar = 100 nm.

home to it, incorporate into the DEJ, and improve epidermal–dermal adherence.

To determine whether IV rC7 could restore AFs at the DEJ of the engrafted RDEB mouse skin *in vivo*, we carried out immuno-electron microscopy using a monoclonal antibody that is specific to human C7 and recognizes an epitope within the NC1 domain of C7, as described (Sakai *et al.*, 1986; Sakai and Keene, 1994). As shown in Figure 2b, the injected rC7 incorporated into the DEJ of the engrafted RDEB mouse skin and formed AF structures, thus demonstrating correction of the major ultrastructural abnormality of RDEB mouse skin. By contrast, there were no detectable AFs at the DEJ of RDEB mouse skin grafts transplanted onto mice that received intravenous PBS. These data indicate that protein-based therapy by IV injection of rC7 can correct the abnormal RDEB dermal–epidermal separation and restore C7 expression and AF formation at the DEJ *in vivo*.

In summary, we showed that rC7 administered intravenously to mice homed to engrafted RDEB mouse skin, incorporated into the DEJ of the grafts, and restored C7, AFs, and epidermal–dermal adherence. These data suggest that intravenously administered rC7 could simultaneously migrate to the DEJ throughout the RDEB patient's skin, reverse the ''subclinical'', microscopic epidermal–dermal separation, and prophylactically prevent frank skin blisters and erosions from forming. We believe that protein therapy via intravenous rC7 may be a valid therapeutic strategy for patients with RDEB who currently have few therapeutic options.

CONFLICT OF INTEREST

Dr Mei Chen and Dr David Woodley are consultants for Lotus Tissue Repair, Inc. and hold stock in the company. Dr Mei Chen, Dr David T. Woodley, and the University of Southern California hold patents for recombinant type VII collagen and have filed a Conflict of Interest Declaration with Dr Randoph W. Hall, Vice Provost for Research Advancement at the University of Southern California.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

REFERENCES

Burgeson RE (1993) Type VII collagen, anchoring fibrils, and epidermolysis bullosa. *J Invest Dermatol* 101:252–5

Chen M, Kasahara N, Keene DR *et al.* (2002) Restoration of type VII collagen expression and function in dystrophic epidermolysis bullosa. *Nat Genet* 32:670–5

Christiano AM, Greenspan DS, Lee S *et al.* (1994) Cloning of human type VII collagen: complete primary sequence of the α1(VIII) chain and identification of intragenic polymorphisms. *J Biol Chem* 269:20256–62

Fine JD, Eady RA, Bauer EA *et al.* (2008) The classification of inherited epidermolysis bullosa (EB): report of the Third International Consensus Meeting on Diagnosis and Classification of EB. *J Am Acad Dermatol* 58:931–50

Heinonen S, Männikkö M, Klement JF *et al.* (1999) Targeted inactivation of the type VII collagen gene (Col7a1) in mice results in severe blistering phenotype: a model for recessive dystrophic epidermolysis bullosa. *J Cell Sci* 112:3641–8

Ortiz-Urda S, Lin Q, Green CL *et al.* (2003) Injection of genetically engineered fibroblasts corrects regenerated human epidermolysis bullosa skin tissue. *J Clin Invest* 111: 251–5

Ortiz-Urda S, Thyagarajan B, Keene DR *et al.* (2002) Stable nonviral genetic correction of inherited human skin disease. *Nat Med* 8:1166–70

Remington J, Wang X, Hou Y *et al.* (2009) Injection of recombinant human type VII collagen corrects the disease phenotype in a murine model of dystrophic epidermolysis bullosa. *Mol Ther* 17:26–33

Saelman EU, Nieuwenhuis HK, Hese KM *et al.* (1994) Platelet adhesion to collagen types I through VIII under conditions of stasis and flow is mediated by GPIa/IIa (alpha 2 beta 1-integrin). *Blood* 83:1244–50

Sakai LY, Keene DR (1994) Fibrillin: monomers and microfibrils. In: Ruoslahti E, Engvall E (eds). *Methods in Enzymology* volume 245. Academic Press: San Diego, CA, pp 47–50

Sakai LY, Keene DR, Morris NP *et al.* (1986) Type VII collagen is a major structural component of anchoring fibrils. *J Cell Biol* 103: 1577–86

Wagner JE, Ishida-Yamamoto A, McGrath JA *et al.* (2010) Bone marrow transplantation for recessive dystrophic epidermolysis bullosa. *N Engl J Med* 363:629–39

Wong T, Gammon L, Liu L *et al.* (2008) Potential of fibroblast cell therapy for recessive dystrophic epidermolysis bullosa. *J Invest Dermatol* 128:2179–89

Woodley DT, Keene DR, Atha T *et al.* (2004) Injection of recombinant human type VII collagen restores collagen function in dystrophic epidermolysis bullosa. *Nat Med* 10:693–5

Woodley DT, Keene DR, Atha T *et al.* (2004) Intradermal injection of lentiviral vectors corrects regenerated human dystrophic epidermolysis bullosa skin tissue *in vivo*. *Mol Ther* 10:318–26

Woodley DT, Krueger GG, Jorgensen CM *et al.* (2003) Normal and gene-corrected dystrophic epidermolysis bullosa fibroblasts alone can produce type VII collagen at the basement membrane zone. *J Invest Dermatol* 121: 1021–8